Toxicity of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) in Aquatic Organisms*

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Herbicides are particularly important in modern forest management as foresters attempt to make fullest use of a constantly shrinking production base. In forestry, (2,4,5,-T) 2,4,5-trichloro-phenoxyacetic acid is used to control undesirable woody species that compete with more desirable timber-producing conifers for light, space, moisture, and nutrients. Herbicide applications can result in markedly increased conifer growth, but such applications must not result in degradation of environmental quality (*1*). In evaluating hazards, scientists have focused on the herbicide, but biologically significant contaminants like 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) must also be considered (*2*).

Herbicides may enter streams by several processes. Direct application or drift of spray materials to surface waters will occur only briefly during the application, but they may cause high concentrations of pollutant in streams. Herbicides could also move to streams in mass overland flow during periods of intense precipitation, but this seldom occurs on forest lands because the infiltration capacity of the forest floor is much greater than most rates of precipitation. Leaching through the soil profile is a slow process capable of only moving small amounts of herbicides short distances and offers little potential for serious stream pollution (*3*).

Studies in the Northwest indicate most contamination of forest streams by herbicides results from drift or direct application of chemical to the water surface. Detectable quantities of 2,4-D and 2,4,5-T have not been found in western streams during fall and winter months after spray applications to nearby forest lands the previous spring (*4*). In spray operations involving the use of 2,4,5-T, we expect small amounts of TCDD will enter the water with the herbicide during application. While some information is available on the toxicity of 2,4,5-T to aquatic organisms, little is known about the toxicity of TCDD.

We conducted chronic toxicity tests to assess the hazard to aquatic organisms which may be exposed to TCDD in water or food after the use of 2,4,5-T in forestry (*5*). Some of the toxic characteristics of TCDD in food and water to several major classes of aquatic organisms are reported here.

Materials and Methods

The organisms tested were three species of fish: guppies (*Poecilia reticulutas*), coho or silver salmon (*Oncorhynchus kisutch*),

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Table 1. Summary of test procedures to determine the toxicity of TCDD in aquatic organisms.

Expt. no.[a]	Organism	Container[b]	Water volume, l.	Water temperature, °C	Exposure regime: Level: Ppt in water	Exposure regime: Level: ng/g Bw[c]	Exposure regime: Duration, hr	Total observation period, days	Feeding regime	Experimental design[d]	Number of replications[e]
1[f]	Guppies (10–40 mm)	1-gal WMJ	3	20	0 100 1,000 10,000		120	37	Tubifix worms, *ad lib.* post exposure period	CRD	3 $n=20$
2	Snails (adult and juvenile)	1-gal WMJ	3	23–27	0 200		1152	48	Elodea, OMP[g] *ad lib.* during exposure period	CRD	4 $n=7$
3	Worms (40 mm)	8-in. culture dish	1	23–27	0 200		1176	55	OMP[g] 1/week, during exposure period	CRD	4 $n=20$
4	Mosquito larvae	8-in. culture dish	1	23–27	0 200		408	39	Yeast 2/week, during exposure period	CRD	4 $n=20$
5	Salmon (7.25 g wet weight)	5-gal WMJ	17	12–18	56 100 560 1,000	13.1 23.4 131.3 234.0	24 48 96	76	OMP[g] 3/week, post exposure period	SPF	4 $n=20$
6	Salmon (1.33 g wet weight)	5-gal WMJ	17	12–18	5.6 11.5 28.0 56.0	7.1 14.1 35.7 71.0	24 48 96	33	OMP[g] 3/week, post exposure period	SPF	4 $n=10$
7	Salmon (3.51 g wet weight)	5-gal WMJ	17	12–18	0.056 0.56 5.6 56.0	0.054 0.54 5.4 54.0	24 48 96	59	OMP[g] 3/week, post exposure period	SPF	4 $n=5$
8	Salmon, (TCDD recovery from water, 2.9 g wet weight)	5-gal WMJ	17	12–16	0 50		24 48 96	—	None	CRD	4
9	Rainbow trout	5-gal	18	11–13	2.3[h]	0.0063[i]	672	28	CDH[j] 2/day	CRD	5

(1.6 g oven dry weight)[k]	aquaria	2,300[h] 2,300,000[h]	6.3[i] 6,300[i]		during exposure period	$n=10$

[a] Expts. 1–8 are static water tests. Salmon experiments (5–8) were all combinations of levels and durations of exposure listed.
[b] WMJ = wide-mouth jar.
[c] Nanograms TCDD per gram wet body weight.
[d] CRD = completely randomized; SPF = split plot factorial.
[e] n = beginning number of organisms per treatment per replication.
[f] Data of Norris and Miller (*10*).
[g] OMP = Oregon moist pellet.
[h] Concentration in food (dry weight).
[i] Weight TCDD tank per week, in nanograms.
[j] Casein, dextrose, herring oil, fish ration (*6*).
[k] Dry weight derived from size-weight relationship for young coho salmon (*7*).

rainbow trout (*Salmo gairdineri*); and three aquatic invertebrates: a snail (*Physa* sp.); a worm (*Paranais* sp.); and mosquito larvae (*Aedes aegypti*). Guppies and mosquitoes were obtained from Oregon State University laboratory cultures, salmon and trout from State of Oregon fish hatcheries, and worms and snails from local streams.

The TCDD (98.7% 2,3,7,8-tetrachlorodibenzo-*p*-dioxin) was obtained from the Dow Chemical Company.

Treatment regimes are summarized in Table 1 for each experiment. In static water test 1 with fish, we expressed exposure levels as nanograms (10^{-9} g) TCDD per gram total body weight of organism as well as in parts TCDD per 10^9 parts water. We do not imply the former are specific body burdens of TCDD but rather the amount of chemical in the container relative to fish biomass at the beginning of the experiment. In some cases, the initial TCDD concentration in the water is also given for reference, but these are of limited value in interpreting the static water toxicity test results because the TCDD concentration did not remain constant and cannot be related to organisms exposed in large bodies of water. We found dose–response relationships in fish were more easily expressed in terms of weights of toxicant and organism biomass.

Static Water Toxicity Tests

For static water toxicity tests, animals were acclimatized for at least 48 hr before they were exposed to TCDD in well water in glass containers (Table 2). TCDD in acetone (maximum 0.3 ml acetone/l.) was added slowly and mixed by stirring and vigorous aeration of water. Control organisms were exposed to an equivalent amount of acetone. At the end of the exposure period, the animals were placed in fresh well water containing no dioxin for the duration of the observation period. During the observation period, after TCDD exposure, the fresh water flow rate through the containers with salmon was 3 l./hr. Water for guppies was exchanged once each 14 days. Salmon were weighed at the beginning of each experiment and at death. Guppy body length was measured at death. Oven-dry weights of worms were made at the end of the observation period.

Table 2. Characteristics of test water.

Constituent	Concn level, ppm	
	Static tests	Feeding tests
Calcium	12.0	14.0
Silica	6.9	27.0
Magnesium	7.8	5.1
Sodium	7.7	5.6
Potassium	1.45	0.6
Bicarbonate	79.0	77.0
Carbonate	0.0	0.0
Sulfate	5.1	
Chloride	4.3	4.0
Nitrate	6.1	0.3
Iron	0.03	0.32
Dissolved solids	132.0	95
Hardness	64.0	56.0
Specific conductance, μmko	164.0	132
pH	6.9	7.7

TCDD Recovery in Static Water Toxicity Tests

To determine TCDD recovery from water containing salmon, TCDD, 0 or 900 ng in 3.6 ml acetone, was added to 17 l. of well water containing 10 coho salmon averaging 2.9 g wet weight each. All conditions were as in experiments 5–8 (Table 1). A total of 12 containers were spiked with TCDD. Each container was sampled only once.

Water samples (1.8 l.) were collected 24, 48, and 96 hr after addition of chemical, and TCDD was determined by gas chromatography*. Each sampling time was replicated four times. Samples of water from containers with salmon but no dioxin were also analyzed to verify adequacy of the cleanup procedure.

*Slightly modified from "A tentative method for analysis of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in pond water." Personal communication, 3/12/71, Dow Chemical Company, Midland, Michigan. Details of analytical procedure available on request.

Chronic Oral Toxicity Tests

For determination of chronic oral toxicity, we exposed young rainbow trout daily to various levels of TCDD in their food. TCDD, to 49% saturation in acetone, was added to the oil base of a casein-dextrose-herring oil fish food, slightly modified from Lee et al. (*6*). Acetone was removed from the oil by vacuum evaporation, leaving an average of 0.3% acetone in the dry fish food. The fish food contained 2.3 ppm, 2.3 ppb, or 2.3 ppt TCDD; exposure levels are in Table 1.

Two hundred young rainbow trout selected for uniformity of size were randomly assigned among 20 aquaria which received fresh water at the rate of 9 l./hr. The 20 aquaria were assigned at random among one control and three treatments in five replications. Fish were acclimatized to the aquaria–flowing water systems and TCDD-free food for 3 weeks before beginning the experiment. Preweighed food given daily at 0900 hr contained the daily dose of TCDD. At 1500 hr daily, food without TCDD was given *ad libitum*, and total daily consumption was recorded. To determine growth, fish were photographed once each week, and fish size index (the product of fish length and depth) obtained from a sideview photograph. In other experiments with coho salmon, sideview area was highly correlated with dry weight (*7*). We will establish a similar relationship for rainbow trout and express fish size in dry weight in later reports.

Results and Discussion

TCDD Recovery in Static Water Toxicity Tests

The TCDD level in water with young salmon declined significantly with time (Fig. 1). Regression analysis indicated recovery between 24 and 96 hr was linear with time (*8*):

$$Y = 63.1 - 13.5X$$

where Y is percentage recovery of TCDD, X is time in hours after addition of TCDD to water containing coho salmon; $r^2 = 0.86$.

TCDD concentration decreased more rapidly between 0 and 24 hr than between 24 and 96 hr, which may suggest more than one mechanism of loss was operative. The rapid loss of TCDD during the first 24 hr may largely be the result of adsorption phenomena which rapidly attain equilibrium. This hypothesis is supported by results from a similar test in which fish were not included and TCDD recovery was 60.0% 4 hr after addition of the chemical.

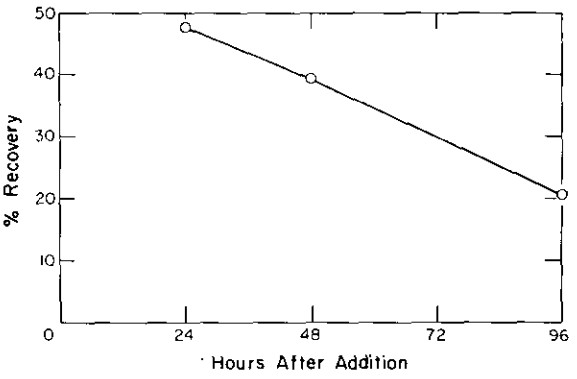

FIGURE 1. Average recovery of TCDD from water spiked with 50 ppt TCDD and containing 10 young coho salmon (four replications).

The fate of TCDD in the system is not known, but we suspect a combination of uptake by fish, adsorption on glass and suspended organic matter, and possibly loss due to aeration. Organisms in our static water toxicity tests were exposed to rapidly declining levels of TCDD because exposure solutions were not replenished. The exposure levels in Table 1 are the initial exposure levels, no adjustment being made for possible changes in TCDD concentration with time.

Fish Symptoms after TCDD Exposure

A difficulty in studying the toxicity of TCDD to fish is that the response to the chemical is not immediate. In most static water test procedures, observations would have been terminated after 96 hr (*9*). In our tests, initial response to the chemical did not occur for 5 to 10 days after the beginning of the exposure period, and mortality often extended over the next 2 months.

Fish exposed to toxic levels of TCDD in water or food showed a declining interest in feeding. Salmon reduced feeding 8 days after TCDD exposure, while guppies responded in 5 days. Affected animals often spit food out shortly after taking it in. Growth of salmon exposed to TCDD in water was markedly inhibited (Fig. 2).

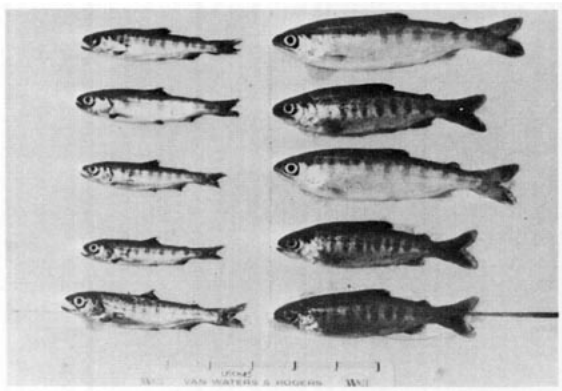

FIGURE 2. TCDD—exposed (13.1 ng/g, 96 hr) and control young coho salmon 80 days after beginning of exposure period.

Skin discoloration and fin necrosis began to appear 15 and 30 days after initial exposure of guppies and salmon, respectively (Fig. 2). Complete loss of the caudal fin occurred in both guppies and salmon. Areas showing skin discoloration often became the site of attack for disease organisms. In salmon, large fungal growths completely encircled some animals and inhibited swimming. Erosion of the upper jaw was seen in guppies surviving 1 to 2 months after exposure but not in salmon. Prior to death, fish often remained close to the bottom of the test containers and showed very little movement. There was no definite pattern prior to death; some fish that appeared perfectly healthy one day were dead the next day while other apparently diseased individuals remained alive for weeks. We detected no differences in behavior between treated and control invertebrate organisms.

Toxicity of TCDD in Water to Young Coho Salmon

Effect of level and duration of exposure—The deaths among exposed salmon frequently did not occur for 10 days after the beginning of the exposure period, regardless of exposure level (Fig. 3). In experiment 5, the effects of exposure to more than 23 ng TCDD/fish wet weight (23 ng/g) for 24 hr was irreversible, and most fish died within 60 days. The effects of level of exposure were quite marked while the effects of duration of exposure were less prominent.

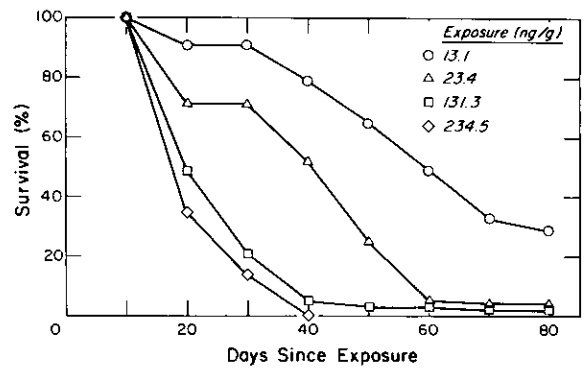

FIGURE 3. Survival of young coho salmon (experiment 5) after exposure to TCDD in water. Values are means for 24, 48, and 96 hr exposure (four replications).

In experiment 7, smaller salmon were used as we attempted to identify the minimum threshold response level for TCDD in water (Table 1). The pattern of delayed mortality observed in experiment 5 was also prominent in experiment 7 (Fig. 4). Exposure to TCDD levels of 54 ng/g for 24 hr or longer was irreversible and killed all fish within 40 days. Exposure to 5.4 ng/g resulted in 55% mortality during the 60-day observation period. Levels of TCDD as low as 0.054 ng/g caused 12% mortality in the 60-day exposure period compared to 2% mortality on controls. It appears these lower levels may be approaching the minimum threshold-response level. The duration of exposure appears less important than levels of exposure in determining mean survival time (Fig. 5).

For statistical analysis, data were expressed as days to death and subjected to multivariate analysis of variance. Mean survival time was significantly reduced with increasing TCDD exposure levels in experi-

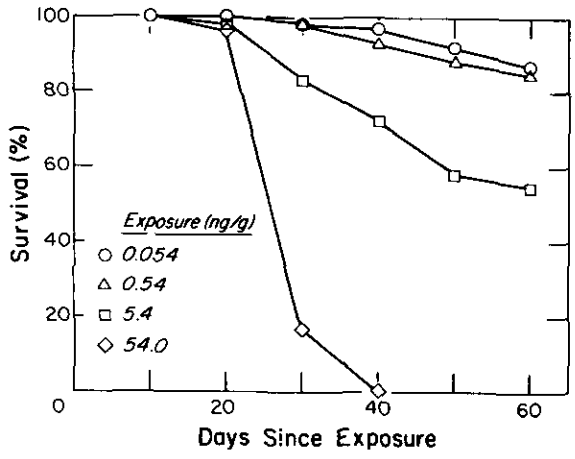

FIGURE 4. Survival of young coho salmon (experiment 7) after exposure to TCDD in water. Values are means for 24, 48, and 96-hr exposure (four replications).

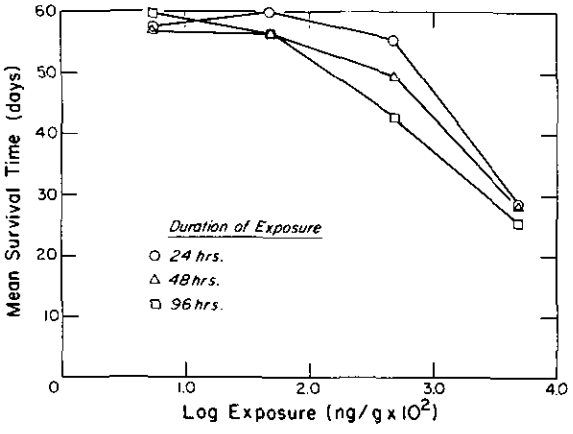

FIGURE 5. Influence of duration of exposure to TCDD on mean survival time of young coho salmon (experiment 7) (four replications).

ments 5, 6, and 7 ($P < 0.01$) Figs. 3 and 4). The duration of exposure effect was less marked, but was significant in experiments 5, 6, and 7 ($P < 0.05$). The duration of exposure-concentration interaction was not significant in any experiment. We feel the duration of exposure effect in salmon may be more pronounced as the minimum threshold–response level is approached and as the duration of exposure is reduced. A duration of exposure effect was not observed in guppies, but the levels of exposure were 2–20 times as great as with coho salmon in experiment 5.

Effect of size of fish on survival time—In both salmon and guppies, larger fish survived for longer periods than smaller fish after TCDD exposure. In some earlier work (*10*), mean survival time was plotted as a function of body length for TCDD exposed guppies ranging from 10 to 40 mm in length (Fig. 6). The regression equation was linear and highly significant ($P < 0.01$). Body length accounted for 93% of the variation of the dependent variable. A similar effect was observed in salmon when data from experiments 5, 6, and 7 were combined (Fig. 7). Time to 50% mortality for salmon exposed to 10 ng for 96 hr was determined graphically for each experiment. Regression analysis showed that the effect of body weight on survival time was linear and significant ($P < 0.01$):

$$Y = 13.8 + 7.7\,X$$

where Y is time, in days, to 50% mortality, X is body wet weight, in grams; $r^2 = 0.87$. Similar responses have been reported for other toxicants (*11*). The ability to tolerate environmental stresses increases with increasing body mass and age, up to a point, in many organisms. Toxicant uptake, storage, and detoxification probably change with fish age, lipid levels, and gill surface area–body mass ratios (*12*).

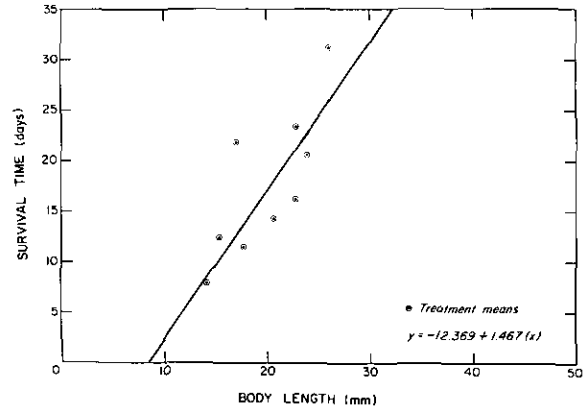

FIGURE 6. Effect of body length on mean survival time of guppies exposed to 100, 1,000, and 10,000 ppt TCDD for 120 hr (*10*) (three replications).

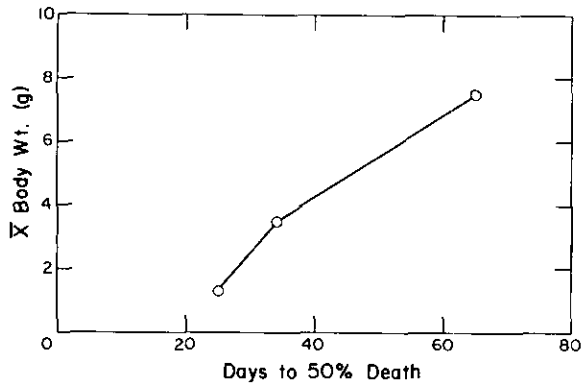

FIGURE 7. Effect of body weight on time to 50% mortality in young coho salmon exposed to 10 ng TCDD per gram wet body weight. Values are means for 24, 48, and 96 hr exposure (four replications).

Toxicity of TCDD in Water to Invertebrate Aquatic Organisms

In these tests, we exposed representatives from the class Insecta, a mosquito larvae; the class Oligochaeta, a worm; and the class Gastropoda, a pulmonate snail to TCDD in static water toxicity tests.

Toxicity to mosquito larvae—In tests with mosquitoes, we observed the maturation of larvae from the second instar through pupation during and after 17-day exposure in water which originally contained 0 or 0.2 ppb TCDD. There were no significant differences in total pupation or the rate of pupation among treated and control mosquitoes during the 30-day test period (Fig. 8).

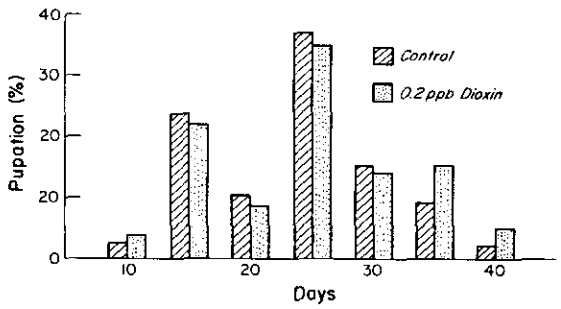

FIGURE 8. Pupation of mosquitoes exposed to 0 or 200 ppt TCDD for 17 days in water (four replications).

Toxicity to snails—Adult pulmonate snails deposited numerous egg cases in containers of well water which originally contained 0 or 0.2 ppb TCDD during a 36-day exposure period. Snail eggs completed development in the original exposure solution, and live juvenile snails and empty juvenile snail shells were counted 48 days after the beginning of the experiment. There was no significant difference between the survival of treated and control adult snails (Fig. 9).

Differences in the total snail hatch between treated and control organisms were observed in each replication, but variation among replications reduced the statistical sensitivity of these tests ($P=0.056$). Differences in the percentage survival of young snails were not significant. TCDD appeared to have its major impact on the reproductive success of snails rather than on survival of either adult or juvenile forms, in that the major effect was on total number of juvenile snail shells (Fig. 9).

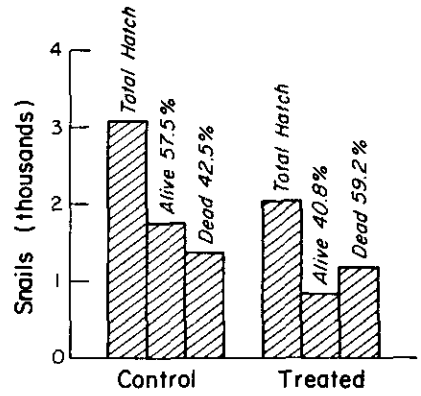

FIGURE 9. Total hatch and survival of juvenile snails from egg masses deposited in 0 or 200 ppt TCDD in water during a 36-day adult snail exposure period. Counts were made 48 days after the beginning of the exposure period.

Toxicity to aquatic worms—Adult Oligochaete worms were exposed to 0 or 0.2 ppb TCDD in water for 55 days. Animals were counted at 30, 48, and 55 days after the beginning of the exposure period. At 55 days, total and mean dry weights were determined.

Table 3. Toxicity of TCDD in water to Oligochaete worms.

TCDD, ppt	Number of worms				Biomass, mg dry wt	
	Initial	30 days	48 days	55 days	Total at 55 days	Mean individual at 55 days
0	80	233	409	414	374	0.90
200	80	195	310	266	193	0.73

Exposure of worms to TCDD resulted in a decrease in the total number of worms present at the end of the 55-day exposure period ($P < 0.05$) (Table 3). Reductions in total worm biomass between treated and control organisms occurred in each replication, but variation among replications reduced the statistical sensitivity of this test ($P = 0.057$). TCDD exerted its principal effect on reproduction rather than growth of individual worms.

Toxicity of TCDD in Food to Young Rainbow Trout

Young rainbow trout (10/aquaria) were exposed to 0, 6.3 pg, 6.3 ng, or 6.3 μg TCDD per tank per week in food (Table 1). The TCDD-containing ration was offered each morning, and TCDD-free food was offered each afternoon. Survival was tallied daily, and growth was measured weekly.

There were no deaths among fish exposed to TCDD in the first 28 days of the experiment, but deaths began to occur in fish exposed to 6.3 μg TCDD per tank per week after 33 days of exposure. The appetite of fish receiving this dose began to decline after 10 days, and by 14 days fin necrosis was apparent. No loss of appetite or fin erosion occurred in fish exposed to lower levels of TCDD.

We observed no differences in the growth of fish receiving 0, 6.3 pg, or 6.3 ng TCDD per tank per week during the first 28 days of the experiment (Fig. 10). The growth of fish receiving 6.3 μg TCDD per week departed markedly from the others after 7 days, and they lost weight for the remaining 21 days of the exposure period. The data, expressed as the fish size index (product of fish length and body depth) were subjected to analysis of variance to test for differences among fish size after 28 days of exposure. There were no differences in the size of fish receiving 0, 6.3 pg, or 6.3 ng TCDD per tank per week. The difference between this group of fish and fish receiving 6.3 μg TCDD per tank per week was highly significant ($P < 0.01$).

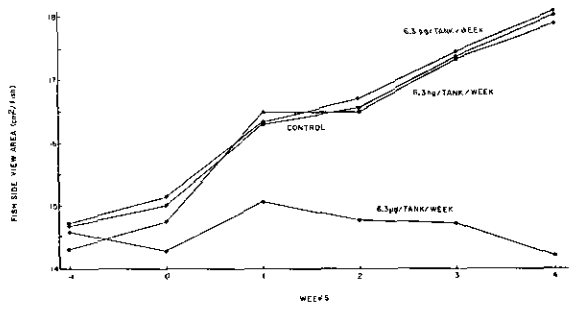

FIGURE 10. Average fish size index (length × body depth) of rainbow trout receiving TCDD in food daily (five replications).

Our data indicate TCDD in food can cause growth reduction and mortality in fish. The oral threshold response level for exposure periods up to 28 days is greater than 6.3 ng TCDD per tank per week. Additional experimentation is necessary to define more precisely the oral threshold response levels and to determine the impact of long-term chronic exposure in food to fish.

TCDD Residues in the Forest

TCDD residues have not been reported in either terrestrial or aquatic components of the forest, but we are not aware of any serious sampling efforts. 2,4,5-T and other herbicides have been reported in Northwest forest streams (*4*). Existing stocks of 2,4,5-T may contain up to 0.5 ppm TCDD, but new formulations must contain less than 0.1 ppm TCDD. We calculated the levels of TC-

DD which might be in forest streams after the aerial application of 2,4,5-T assuming the principal route of entry was drift or direct application of spray materials to the stream surface. Levels of 2,4,5-T are not expected to exceed 0.1 ppm if applications are carefully controlled (Table 4). A more complete determination of threshold response levels will be required, however, before an adequate assessment of TCDD hazard to stream organisms can be made.

Conclusions

TCDD in water or food is toxic to fish. The effects of exposure for 24–96 hr of young salmon to TCDD in water at levels greater than 23 ng/g is irreversible, and death results in 10–80 days. Duration of exposure is less important than level of exposure except as threshold response levels are approached. The critical exposure period may be somewhat less than 24 hrs in static water toxicity tests in which TCDD concentration may change markedly with time. Small fish are more sensitive than large fish on an equivalent exposure level basis. TCDD in food at 2.3 ppm markedly reduced growth of young rainbow trout (10/aquaria) exposed to 6.3 μg TCDD per tank per week for 4 weeks. TCDD at 0.2 ppb had no effect on pupation of mosquito larvae, but reduced the reproductive success of a pulmonate snail and an Oligochaete worm.

Table 4. TCDD in streamwater after aerial application of 2,4,5-T to forest land.

2,4,5-T in streamwater, ppm	Anticipated TCDD in streamwater, ppt	
	Level 1 [a]	Level 2 [b]
1.0	0.5	0.1
0.1	0.05	0.01
0.05	0.025	0.005
0.01	0.005	0.001
0.005	0.0025	0.0005

[a] Level 1: 2,4,5-T contains 0.5 ppm TCDD.
[b] Level 2: 2,4,5-T contains 0.1 ppm TCDD.

Our research has established some important toxicity characteristics of TCDD in fish, but considerable work remains to be done. Establishment of minimum threshold response levels during long- and short-term exposure are important. The impact of previous and current TCDD exposure on long-term growth and reproduction of fish needs attention. Information on its movement, persistence, and fate of TCDD in aquatic systems will be required to adequately assess the impact of TCDD in streams. Serious attempts to determine TCDD residues in various parts of the natural aquatic ecosystem are badly needed. The most sensitive analytical techniques and positive means of residue identification will be necessary.

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